K092333

OCT 27 2010

SECTION 3 – 510(k) SUMMARY OF SAFETY AND EFFECTIVENESS

K092333

OCT 27 2010

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR §807.92(c)

Submitted by:
Indu Lakshman, Director of Quality & Regulatory Affairs
BioImagene, Inc
919 Hermosa Ct. Sunnyvale, CA 94085 United States

Date summary prepared: June, 2009
Date summary updated: Oct, 2009

Trade Name: PATHIAM™ System with iScan for p53 and Ki-67

Classification Name: Microscope, automated, image analysis, immunohistochemistry, operator intervention, nuclear intensity & percent positivity.

Device Description:
The PATHIAM™ System is an instrument and software system designed to assist the qualified pathologist in the consistent quantitative assessment of protein expression in immunohistochemically stained histologic sections from formalin-fixed, paraffin-embedded normal and neoplastic tissues. The system consists of a slide scanner (iScan), computer, monitor, keyboard, mouse, image analysis algorithms for specific immunohistochemical markers, and software with a Windows web browser-based user interface. PATHIAM is a web-based, end-to-end digital pathology software solution that allows pathology labs to acquire, manage, view, analyze, share, and report on digital images of pathology specimens. Using the PATHIAM software, the pathologist can view digital images, add annotations, make measurements, perform image analysis, and generate reports.

Hardware: The iScan slide scanning device captures digital images of formalin-fixed, paraffin-embedded tissues that are suitable for storage and viewing. The device includes a digital slide scanner, racks for loading glass slides, computer, scanner software, keyboard, mouse and monitor.

Software: The PATHIAM software is designed to complement the routine workflow of a qualified pathologist in the review of immunohistochemically stained histologic slides. It allows the user to select fields of view (FOVs) in the digital image for analysis and provides quantitative data on these FOVs to assist with interpretation. The software makes no independent interpretations of the data and requires competent human intervention at all steps in the analysis process.

Indications for Use:
The p53 results provided by the PATHIAM System are indicated for use ~~on is a useful tool~~ for the identification of p53 accumulation in human neoplasias when used with IVD reagents marketed for this indication. Interpretation should be made within the context of the patient's

clinical history and other diagnostic tests by a qualified pathologist. The pathologist must verify agreement with the PATHIAM score.

Ki-67 results provided by the PATHIAM System are indicated for use to assess proliferative activity when used with in vitro diagnostic reagents marketed for this indication. Interpretation should be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. The pathologist must verify agreement with the PATHIAM score.

Predicate Device:
Tripath Imaging, Inc.
Ventana® Image Analysis System (VIAS™)
K062428 – VIAS p53 application
K053520 – VIAS Ki-67 application
Regulation: 21 CFR §864.1860, Immunohistochemistry Reagents and Kits
Product Code: NQN
Panel: Pathology

Performance:

PATHIAM System Comparison Studies (Inter and Intra Pathologist Studies)

Inter pathologist study

Round 1 Manual Scoring:

Slides were scored by a qualified pathologist at each site manually. The three pathologists read randomly selected 120 stained tissue test samples manually on a microscope and assigned a score to each specimen (test sample) according to the scoring categories.

Round 2 PATHIAM Assisted Scoring:

PATHIAM assisted scoring took place after a minimum of one week passed since manual slide reading. The order that the test samples were accessed (randomized) for scoring was presented to the pathologists at the time the testing was administered and was different from the order presented in Round 1 to further reduce the possibility that the manual scoring influenced the scoring using the PATHIAM system. The same three pathologists reviewed the digital images of the test samples presented by the software on the computer monitor (PATHIAM system). The pathologist had the ability to navigate freely around the images at various magnifications (as in a microscope), select field of views for scoring, and determine the score for each specimen (test sample) with the assistance of the Pathiam system according to the scoring categories.

The above two steps (Round 1 and Round 2) were performed with three investigators on the same set of test samples.

Table 1: Concordance Results for p53 Scoring

p53 Cut-Off Threshold	Manual vs PATHIAM-assisted Substantial Equivalence Concordance Range for 3 Pathologists	PATHIAM-assisted vs PATHIAM-assisted Reproducibility Concordance Range for 3 Pathologists	Manual vs Manual Reproducibility Concordance Range for 3 Pathologists
>1%	82% - 90%	88% - 93%	78% - 95%
>5%	77% - 85%	90% - 93%	78% - 88%
>10%	83% - 89%	93% - 97%	86% - 90%

Table 2: Concordance Results for Ki-67 Scoring

Ki-67 Cut-Off Threshold	Manual vs. PATHIAM-assisted Substantial Equivalence Concordance Range for 3 Pathologists	PATHIAM-assisted vs. PATHIAM-assisted Reproducibility Concordance Range for 3 Pathologists	Manual vs. Manual Reproducibility Concordance Range for 3 Pathologists
>1%	88%-93%	92%-94%	86%-91%
>5%	87%-93%	90%-93%	85%-89%
>10%	81%-89%	88-95%	80%-91%

PATHIAM System Reproducibility and Precision Study (Inter and Intra System Studies)

The intra system (PATHIAM system with iScan) study was performed on five sets of images (one set = eight test samples) produced by one scanner and scored on one computer system (consisting of a computer, monitor, keyboard, p53 & Ki-67 image analysis algorithms, MS Windows web browser and a mouse). This study was repeated on a total of three different scanners and computer systems. Test samples were pre-selected (field of views) by a qualified pathologist. See the data analysis tables below.

p53 System Study Precision (between run) Results:

Table 5: Intra-system Precision Study – System I for p53

P53 Precision Study – System 1 (n=5)

Sample ID	Mean	SD	%CV
A7	0.00	0.00	-
E3	0.00	0.00	-
C9	42.90	0.02	0.06
B5	2.82	0.08	2.67
E3	73.50	0.05	0.07
B9	16.44	0.01	0.09
D4	22.14	0.07	0.32
B3	24.05	0.06	0.23

Table 6: Intra system Precision Study – System II for p53

P53 Precision Study – System 2 (n=5)

Sample ID	Mean	SD	%CV
A7	0.00	0.00	-
E3	0.00	0.00	-
C9	42.74	0.02	0.05
B5	2.57	0.01	0.58
E3	72.89	0.04	0.06
B9	16.51	0.04	0.24
D4	22.44	0.04	0.17
B3	22.68	0.06	0.25

Table 7: Intra system Precision Study – System III for p53

P53 Precision Study – System 3 (n=5)

Sample ID	Mean	SD	%CV
A7	0.00	0.00	-
E3	0.00	0.00	-
C9	42.60	0.05	0.11
B5	2.71	0.02	0.78
E3	74.07	0.13	0.18
B9	16.49	0.03	0.18
D4	24.42	0.01	0.05
B3	24.90	0.10	0.40

Ki-67 System Study Precision (between run) Results:

Table 8: Intra system Precision Study – System I for Ki-67

	Sample ID	Mean	SD	%CV
Ki67 Precision Study - System 1 (n=5)	A2	31.78	0.10	0.31
	E2	64.53	0.25	0.39
	A3	15.45	0.15	0.99
	D4	17.82	0.09	0.50
	E7	9.76	0.02	0.22
	D6	4.85	0.02	0.40
	E5	9.13	0.12	1.35
	A1	0.88	0.02	1.78

Table 9: Intra system Precision Study – System II for Ki-67

	Sample ID	Mean	SD	%CV
Ki67 Precision Study - System 2 (n=5)	A2	32.77	0.37	1.13
	E2	63.29	0.08	0.12
	A3	15.76	0.17	1.09
	D4	17.91	0.04	0.23
	E7	9.41	0.04	0.44
	D6	4.87	0.14	2.90
	E5	9.27	0.04	0.42
	A1	0.85	0.01	0.89

Table 10: Intra system Precision Study – System III for Ki-67

	Sample ID	Mean	SD	%CV
Ki67 Precision Study – System 3 (n=5)	A2	31.53	0.19	0.59
	E2	62.11	0.23	0.36
	A3	15.05	0.12	0.78
	D4	17.66	0.02	0.14
	E7	9.81	0.07	0.72
	D6	4.95	0.03	0.68
	E5	9.43	0.02	0.24
	A1	0.86	0.00	0.35

Reproducibility (between Run/Inter System) Study

The data from the above three intra-system studies were used to understand the inter-system comparison.

Table 11: Inter system Reproducibility Study – p53

	Sample ID	Mean	SD	%CV
P53 Inter-System Reproducibility - System 1, 2, 3 (n=3x5)	A7	0.00	0.00	-
	E3	0.00	0.00	-
	C9	42.75	0.13	0.30
	B5	2.70	0.12	4.32
	E3	73.49	0.50	0.68
	B9	16.48	0.04	0.25
	D4	23.00	1.05	4.55
	B3	23.88	0.95	3.97

Table 12: Inter system Reproducibility Study – Ki67

Ki67 Inter-System Reproducibility - System 1, 2, 3 (n=3x5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A2	32.03	0.60	1.87
TMA 3 2007	E2	63.31	1.04	1.65
TMA 3 2007	A3	15.42	0.33	2.14
TMA 4 2007	D4	17.79	0.12	0.66
TMA 3 2007	E7	9.66	0.19	1.95
TMA 5 2007	D6	4.89	0.09	1.84
TMA 3 2007	E5	9.28	0.14	1.53
TMA 2 2007	A1	0.86	0.02	2.07

Substantial Equivalence

Table 13: Comparison to Predicate Devices to Support Substantial Equivalence Determination for p53 Image Analysis Systems

Attribute	**PATHIAM System for p53**	**Tripath (VIAS p53) K062428**
Intended Use	This device is intended for in vitro diagnostic (IVD) use. The PATHIAM System is intended as an aid to the pathologist to detect, count, and classify cells of clinical interest based on recognition of cellular objects of particular color, size, and shape, using appropriate controls to assure the validity of the scores. The p53 application is intended for	This antibody is intended for *in vitro* diagnostic (IVD) use. Ventana® Medical Systems (Ventana) CONFIRM anti-p53 (DO-7) primary antibody is a mouse monoclonal antibody (IgG1, kappa) directed against human p53. The antibody is intended for laboratory use to qualitatively identify by light microscopy wild type and mutant p53 in sections of formalin fixed,

Attribute	PATHIAM System for p53	Tripath (VIAS p53) K062428
	use as an aid to the pathologist to quantify the percentage of positively stained nuclei in formalin fixed paraffin embedded breast tissue specimens stained with Dako mouse monoclonal anti-human p53 antibody, clone DO7and visualized with DAB chromogen, to detect both wild-type and mutant p53, a nuclear protein, as specified in the instructions for these reagents. It is the responsibility of a qualified pathologist to employ appropriate morphological studies and controls as specified in the instructions for Dako p53 to assure the validity of the PATHIAM-assisted p53 assessment.	paraffin embedded tissue on a Ventana automated slide stainer.
Indications for use	The p53 results provided by the PATHIAM System are indicated for use for the identification of p53 accumulation in human neoplasias when used with IVD reagents marketed for this indication. Interpretation should be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. The pathologist must verify agreement with the PATHIAM score.	The ***Ventana Image Analysis System*** (VIAS™) is an adjunctive computer-assisted image analysis system functionally connected to an interactive microscope. It is intended for use as an aid to the pathologist in the detection, classification and counting of cells of interest 2 based on marker intensity, size and shape using appropriate controls to assure the validity of the VIAS scores.
Specimen Type	Formalin-fixed, paraffin embedded breast cancer specimens stained by immunohistochemistry reagent for p53	Same
Image Analysis System	Histologic observation by a pathologist through the BioImagene's PATHIAM image analysis system with iScan slide scanner.	Histologic observation by a pathologist through a specified interactive microscope/digital camera with image analysis software.
Hardware and Software	PATHIAM software, BioImagene iScan slide scanner, computer,	VIAS with software, computer, microscope, motorized stage, digital

Attribute	PATHIAM System for p53	Tripath (VIAS p53) K062428
Components	mouse, keyboard, windows web browser and monitor.	color video camera, mouse, keyboard, and monitor.
Assay used	The tissues were stained using the Dako p53, clone DO7™ monoclonal antibody.	Ventana Confirm™ anti-p53 (DO-7)

Table 14: Comparison to Predicate Devices to Support Substantial Equivalence Determination for Ki-67 Image Analysis Systems

Attribute	PATHIAM System for Ki-67	Tripath (VIAS Ki-67) K053520
Intended Use	This device is intended for in vitro diagnostic (IVD) use. The PATHIAM System is intended as an aid to the pathologist to detect, count, and classify cells of clinical interest based on recognition of cellular objects of particular color, size, and shape, using appropriate controls to assure the validity of the scores. The Ki-67 application is intended as an aid to the pathologist to quantify the percentage of positively stained nuclei in formalin-fixed paraffin embedded normal and neoplastic breast tissue specimens immunohistochemically stained with Dako mouse monoclonal anti-human Ki-67 antigen, clone MIB1 visualized with DAB chromogen as specified in the instructions for these reagents. It is the responsibility of a qualified pathologist to employ appropriate morphological studies and controls as specified in the instructions for Dako Ki-67 to assure the validity of the	This device is intended for *in vitro* diagnostic (IVD) use. The ***Ventana Image Analysis System*** (VIAS) is an adjunctive computer-assisted image analysis system functionally connected to an interactive microscope. It is intended for use as an aid to the pathologist in the detection, classification and counting of cells of interest based on marker intensity, size and shape using appropriate controls to assure the validity of the VIAS scores. In this application, the VIAS is intended to aid a qualified pathologist in the acquisition and measurement of images to quantify the percentage of positively stained nuclei in paraffin embedded breast cancer tissue specimens immunohistochemically stained for the presence of Ki-67 proteins using Ventana's reagents and nuclear hematoxylin. It is indicated for use in assessing the proliferative activity of normal and neoplastic breast tissue when used with in vitro

Attribute	PATHIAM System for Ki-67	Tripath (VIAS Ki-67) K053520
	PATHIAM-assisted Ki-67 assessment.	diagnostic reagents marketed for these indications.
Indications for use	Ki-67 results provided by the PATHIAM System are indicated for use to assess proliferative activity when used with in vitro diagnostic reagents marketed for this indication. Interpretation should be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. The pathologist must verify agreement with the PATHIAM score.	It is indicated for use in assessing the proliferative activity of normal and neoplastic breast tissue when used with in vitro diagnostic reagents marketed for these indications
Specimen Type	Formalin-fixed, paraffin embedded specimens stained by immunohistochemistry reagent for Ki-67	Same
Image Analysis System	Histologic observation by a pathologist through the BioImagene's PATHIAM image analysis system with/ iScan slide scanner.	Histologic observation by a pathologist through a specified interactive microscope/digital camera with image analysis software.
Hardware and Software Components	PATHIAM software, BioImagene iScan slide scanner, computer, mouse, keyboard, windows web browser and monitor.	VIAS with software, computer, microscope, motorized stage, digital color video camera, mouse, keyboard, and monitor.
Assay used	The tissues were stained using Dako Ki-67, clone MIB1 antibody.	Per Ventana Ki-67 kit product insert (Catalogue Number 790-2910)

Standards Employed

None under Section 514

FDA Guidance

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, May 11, 2005

SECTION 4 – DEVICE DESCRIPTION

General Description

The PATHIAM™ System is an instrument and software system designed to assist the qualified pathologist in the consistent quantitative assessment of protein expression in immunohistochemically stained histologic sections from formalin-fixed, paraffin-embedded normal and neoplastic tissues. The system consists of a slide scanner (iScan), computer, monitor, keyboard, mouse, image analysis algorithms for specific immunohistochemical markers, and software with a Windows web browser-based user interface. PATHIAM is a web-based, end-to-end digital pathology software solution that allows pathology labs to acquire, manage, view, analyze, share, and report on digital images of pathology specimens. Using the PATHIAM software, the pathologist can view digital images, add annotations, make measurements, perform image analysis, and generate reports.

Hardware: The iScan slide scanning device captures digital images of formalin-fixed, paraffin-embedded tissues that are suitable for storage and viewing. The device includes a digital slide scanner, racks for loading glass slides, computer, scanner software, keyboard, mouse and monitor.

Software: The PATHIAM software is designed to complement the routine workflow of a qualified pathologist in the review of immunohistochemically stained histologic slides. It allows the user to select fields of view (FOVs) in the digital image for analysis and provides quantitative data on these FOVs to assist with interpretation. The software makes no independent interpretations of the data and requires competent human intervention at all steps in the analysis process.

Additional materials required:

- Dako p53, clone DO7™ monoclonal antibody
- Dako Ki-67, clone MIB1 monoclonal antibody
- Reagents for visualization, such as DAB chromagen
- Associated materials for completing immunohistochemical staining according to the appropriate package insert
- Color printer if user wishes to print out color copies

Device Quality Control

The quality of results depends on the laboratory following the quality control instructions recommended in the labeling of the immunohistochemistry (IHC) reagents. The software also performs a quality check on the digital images to determine if they are suitable for further analysis using "Image Quality Assessment" algorithms.

Summary of Procedure

Samples are obtained as formalin-fixed, paraffin-embedded tissue blocks. Histologic sections are prepared and mounted onto glass slides. Slides are reacted with either Ki-67 or p53 primary antibodies. Slides are visualized using DAB. Prepared slides are loaded into the PATHIAM system scanner and scanned. The resulting digital images are reviewed by the pathologist on a computer monitor, and appropriate fields of view (FOVs) are then selected for analysis by the PATHIAM software. The PATHIAM software produces a "percent positive" result for the specific immunohistochemical study (Ki-67 or p53), and the pathologist has the choice of accepting the result or entering his/her own score.

Principal of Operation

After the initial image quality check, the algorithm goes through the following steps before generating the analysis results:

1. **Field of View (FOV) identification**: The algorithm separates the tissue area from the background such that only the tissue area is processed in the following steps.
2. **Preprocessing:** The algorithm generates two images after preprocessing. One of them is a contrast stretched image, and the other is an image with each of the tissue AOI pixels classified as stained or non-stained.
3. **Segmentation:** This processing step consists of extracting the objects of interest from the image. In the current applications, the objects of interest are epithelial cell nuclei. These are separated out from the rest of the identified objects using morphological properties, such as size and shape.
4. **Classification:** The segmented nuclei are classified as stained cells or non-stained cells based on the percentage of stained pixels within them.
5. **Scoring / Grading:** Based on the classification, an overall score for the image is computed using the numbers of stained cells, non-stained cells and total cells for the calculations.

Table 15 - BioImagene iScan Slide Scanner Specifications

Input Format	1 x 3 inch (25 x 75mm) microscope slides
Slide Capacity	1 to 160 slides using 8 integrated standard Sakura racks
Microscope Objective	Olympus 20x/0.50 Plan Fluor (Nikon 20x/0.50 Plan Fluor)
Scanning Resolution	0.46 µm/pixel @ 20x
Camera Frame Size	1392x1032
Light Source (Illumination)	Integrated LED
Auto-Scan	Automated barcode reading, tissue identification, autofocus, scanning and JPEG 2000 compression for up to 160 slides
Manual Scan	User selects scan area for single or batched slides in automatic or manual mode
Throughput	~ 4 minutes/slide in batch mode (15 x 15mm scan area @ 20x) - Time To View (defined as total pre-processing time, scanning time and encoding time)
Scan Viewing	24-bit true color
Slide Storage Format	JPEG 2000
Compression	1:1 – 20:1 (range)
Barcode Capability	1D and 2D option
Dimensions	Approximately 18 x 18 x 17 high inches (45 x 45 x 41 high mm)
Weight	75 lbs (23 kg)
Power	110-240 VAC, 50/60 Hz

SECTION 5 – COMPARATIVE INFORMATION

Substantial Equivalence

Table 16: Comparison to Predicate Devices to Support Substantial Equivalence Determination

Attribute	PATHIAM System	Tripath (VIAS p53) K062428
Intended Use	This device is intended for in vitro diagnostic (IVD) use. The PATHIAM System is intended as an aid to the pathologist to detect, count, and classify cells of clinical interest based on recognition of cellular objects of particular color, size, and shape, using appropriate controls to assure the validity of the scores. The p53 application is intended for use as an aid to the pathologist to quantify the percentage of positively stained nuclei in formalin fixed paraffin embedded breast tissue specimens stained with Dako mouse monoclonal anti-human p53 antibody, clone DO7and visualized with DAB chromogen, to detect both wild-type and mutant p53, a nuclear protein, as specified in the instructions for these reagents. It is the responsibility of a qualified pathologist to employ appropriate morphological studies and controls as specified in the instructions for Dako p53 to assure the validity of the PATHIAM-assisted p53 assessment.	This antibody is intended for *in vitro* diagnostic (IVD) use. Ventana® Medical Systems (Ventana) CONFIRM anti-p53 (DO-7) primary antibody is a mouse monoclonal antibody (IgG1, kappa) directed against human p53. The antibody is intended for laboratory use to qualitatively identify by light microscopy wild type and mutant p53 in sections of formalin fixed, paraffin embedded tissue on a Ventana automated slide stainer.
Indications for use	The p53 results provided by the PATHIAM System are indicated for use for the identification of p53 accumulation in human neoplasias	The ***Ventana Image Analysis System*** (VIAS™) is an adjunctive computer-assisted image analysis system

Attribute	PATHIAM System	Tripath (VIAS p53) K062428
	when used with IVD reagents marketed for this indication. Interpretation should be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. The pathologist must verify agreement with the PATHIAM score.	functionally connected to an interactive microscope. It is intended for use as an aid to the pathologist in the detection, classification and counting of cells of interest based on marker intensity, size and shape using appropriate controls to assure the validity of the VIAS scores.
Specimen Type	Formalin-fixed, paraffin embedded breast cancer specimens stained by immunohistochemistry reagent for p53	Same
Image Analysis System	Histologic observation by a pathologist through BioImagene's PATHIAM image analysis system with iScan slide scanner.	Histologic observation by a pathologist through a specified interactive microscope/digital camera with image analysis software.
Hardware and Software Components	PATHIAM software, BioImagene iScan slide scanner, computer, mouse, keyboard, windows web browser and monitor.	VIAS with software, computer, microscope, motorized stage, digital color video camera, mouse, keyboard, and monitor.
Assay used	Dako p53, clone DO7™ monoclonal antibody	Ventana Confirm™ anti-p53 (DO-7)

Table 17: Comparison to Predicate Devices to Support Substantial Equivalence Determination

Attribute	PATHIAM System	Tripath (VIAS Ki-67) K053520
Intended Use	This device is intended for in vitro diagnostic (IVD) use. The PATHIAM System is intended as an aid to the pathologist to detect, count, and classify cells of clinical interest based on recognition of cellular objects of particular color, size, and shape, using appropriate controls to assure the validity of	This device is intended for *in vitro* diagnostic (IVD) use. The ***Ventana Image Analysis System*** (VIAS) is an adjunctive computer-assisted image analysis system functionally connected to an interactive microscope. It is intended for use as an aid to the pathologist in the detection, classification and

Attribute	PATHIAM System	Tripath (VIAS Ki-67) K053520
	the scores. The Ki-67 application is intended as an aid to the pathologist to quantify the percentage of positively stained nuclei in formalin-fixed paraffin embedded normal and neoplastic breast tissue specimens immunohistochemically stained with Dako mouse monoclonal anti-human Ki-67 antigen, clone MIB1 visualized with DAB chromogen as specified in the instructions for these reagents. It is the responsibility of a qualified pathologist to employ appropriate morphological studies and controls as specified in the instructions for Dako Ki-67 to assure the validity of the PATHIAM-assisted Ki-67 assessment.	counting of cells of interest based on marker intensity, size and shape using appropriate controls to assure the validity of the VIAS scores. In this application, the VIAS is intended to aid a qualified pathologist in the acquisition and measurement of images to quantify the percentage of positively stained nuclei in paraffin embedded breast cancer tissue specimens immunohistochemically stained for the presence of Ki-67 proteins using Ventana's reagents and nuclear hematoxylin. It is indicated for use in assessing the proliferative activity of normal and neoplastic breast tissue when used with in vitro diagnostic reagents marketed for these indications.
Indications for use	Ki-67 results provided by the PATHIAM System are indicated for use to assess proliferative activity when used with in vitro diagnostic reagents marketed for this indication. Interpretation should be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. The pathologist must verify agreement with the PATHIAM score.	It is indicated for use in assessing the proliferative activity of normal and neoplastic breast tissue when used with in vitro diagnostic reagents marketed for these indications
Specimen Type	Formalin-fixed, paraffin embedded specimens stained by immunohistochemistry reagent for Ki-67	Same
Image Analysis System	Histologic observation by a pathologist through BioImagene's PATHIAM image	Histologic observation by a pathologist through a specified interactive microscope/digital

Attribute	PATHIAM System	Tripath (VIAS Ki-67) K053520
	analysis system with iScan slide scanner.	camera with image analysis software.
Hardware and Software Components	PATHIAM software, BioImagene iScan slide scanner, computer, mouse, keyboard, windows web browser and monitor.	VIAS with software, computer, microscope, motorized stage, digital color video camera, mouse, keyboard, and monitor.
Assay used	Dako Ki-67, clone MIB1 monoclonal antibody.	Per Ventana Ki-67 kit product insert (Catalogue Number 790-2910)

Substantial Equivalence Conclusion

Substantial equivalence is demonstrated by identical intended use and similar performance. The technological differences between the device and the predicate do not raise new questions or concerns of safety and effectiveness.

SECTION 6 – PERFORMANCE TESTING

PATHIAM System Comparison Studies (inter and intra pathologist)

Title: Performance of the PATHIAM System for analysis of p53 and Ki-67 nuclear protein immunohistochemistry in breast tissue.

Objective:
The objectives of the study were two-fold:

1. To compare the performance of the PATHIAM system to manual microscopy for the assessment of Ki67 & p53 immunohistochemistry.
2. To determine whether inter-pathologist and intra-pathologist scoring of Ki67 & p53 immunohistochemistry using the PATHIAM system is reproducible.

Protocol Number: TP-000046 Rev. B

Device Description:
The PATHIAM™ System is an instrument and software system designed to assist the qualified pathologist in the consistent quantitative assessment of protein expression in immunohistochemically stained histologic sections from formalin-fixed, paraffin-embedded normal and neoplastic tissues. The system consists of a slide scanner (iScan), computer, monitor, keyboard, mouse, image analysis algorithms for specific immunohistochemical markers, and software with a Windows web browser-based user interface. PATHIAM is a web-based, end-to-end digital pathology software solution that allows pathology labs to acquire, manage, view, analyze, share, and report on digital images of pathology specimens. Using the PATHIAM software, the pathologist can view digital images at various magnifications, add annotations, make measurements, perform image analysis, and generate reports.

Predicates: K053520, K062428

Investigators:
Gist Farr, MD, Spartanburg, SC
Lynn Goldstein, MD, PhenoPath Laboratories, Seattle, WA
Beiru Chen, MD, Delta Pathology Associates, Stockton, CA

Investigator Training: Investigators received training prior to participation in the study. Training included a review of the protocol, good clinical practice, detailed manual scoring instructions, case report form completion, study timelines, and a microscope session that included a reference slide review, use of the sample list, TMA map, and low power print outs.

Immediately prior to the second round of scoring using the PATHIAM system, a second training was conducted that included a review of a PATHIAM Scoring Presentation, detailed PATHIAM Scoring Instructions and a computer session that consisted of reference slide review, use of the sample list, and assisted scoring with the PATHIAM Software.

Sample Procurement Center:
Ohio State University Medical Center
310 Doan Hall, 410 West 10th Av, Columbus, OH
CLIA # 36D1046162

Tissue Procurement, Preparation and Staining:

Procurement and slide preparation was under the direction of Dr. Sanford H. Barsky, Chair, Department of Pathology, Ohio State University School of Medicine (OSU). Tissues were acquired from select patient material in the form of archived pathological specimens stored as either paraffin blocks or previously made Tissue Micro Arrays (TMAs). 188 formalin fixed paraffin blocks of breast cancer samples from different patients were used to prepare five tissue micro-arrays (TMAs). Individual TMA cores measured 2 mm in diameter. Sections from each block were prepared at OSU and mounted onto glass slides.

The slides were stained for the identification of Ki67 protein using Dako clone MIB1 monoclonal antibody and DAB detection and for the identification of p53 protein using Dako clone DO7™ monoclonal antibody and DAB detection.

Comparative Study Investigators:
Gist Farr, MD, Spartanburg, SC
Lynn Goldstein, MD, PhenoPath Laboratories, Seattle, WA
Beiru Chen, MD, Delta Pathology Associates, Stockton, CA

Study Locations:

	Manual Scoring	PATHIAM Scoring
Dr. Farr	University of Puget Sound Biology Dept. 1500 North Warner St. Tacoma, WA 98416	Sound Clinical Research, LLC 3519 N Adams St Tacoma, WA 98407
Dr. Chen	Division of Pathology and Lab Medicine Doctors Hospital 1205 E North St, Manteca, CA 95336	BioImagene, Inc. 919 Hermosa Court Sunnyvale, CA 94085
Dr. Goldstein	PhenoPath Laboratories 551 North 34th Street, Suite 100 Seattle, Washington 98103	PhenoPath Laboratories 551 North 34th Street, Suite 100 Seattle, Washington 98103

IRBs:
For the Use of Tissue:
Ohio State University
Office of Responsible Research Practices
300 Research Foundation
1960 Kenny Road
Columbus, OH 43210-1063

For the Study Protocol and Investigators:
Aspire IRB
9320 Fuerte Drive, Suite 105
La Mesa, CA 91941

Study Design

The Ki67study involved three investigators (qualified pathologists) affiliated with different clinical labs utilizing 120 de-identified archived breast carcinoma sections in TMA form, stained for the identification of Ki67 protein using Dako clone MIB1 monoclonal antibody and DAB detection. Samples spanned a range of positivity from 0 (negative) to 100%. The slides (test samples) required for the study were scanned by BioImagene.

The p53 study involved three investigators (qualified pathologists) affiliated with different clinical labs utilizing 120 de-identified archived breast carcinoma sections in TMA form, stained for the identification of p53 protein using Dako clone DO7™ monoclonal antibody and DAB detection. Samples spanned a range of positivity from 0 (negative) to 100%. The slides (test samples) required for the study were scanned by BioImagene.

System & Input Requirements for PATHIAM System

Application	Computer Analysis of Digitized Image
System Requirements	iScan scanner Computer, Monitor PATHIAM Software
System Input	Microscope slides to scanner
Pathologist Input	Select image to load from scanner Select test samples Score test sample under microscope Choose FOVs from each test sample Click ANALYZE button

	Review PATHIAM Score Accept PATHIAM score or Enter own score (PATHIAM-assisted)

Inter pathologist study

Round 1 Manual Scoring:

Slides were scored by a qualified pathologist at each site manually. The three pathologists individually reviewed 120 tissue samples immunohistochemically stained for p53 or Ki67 on a microscope and assigned a score to each specimen (test sample) according to the scoring categories.

Round 2 PATHIAM Assisted Scoring:

PATHIAM assisted scoring took place after a minimum of one week passed since manual slide reading. The order that the test samples were accessed (randomized) for scoring was presented to the pathologists at the time the testing was administered and was different from the order presented in Round 1 to further reduce the possibility that the manual scoring influenced the scoring using the PATHIAM system. The same three pathologists reviewed the digital images of the test samples presented by the software on a computer monitor (PATHIAM system). The pathologists had the ability to navigate freely around the images at various magnifications (as in a microscope), select fields of views for scoring, and determine the score for each specimen (test sample) with the assistance of the PATHIAM system according to the scoring categories.

The above two steps (Round 1 and Round 2) were performed with three investigators on the same set of test samples.

Intra pathologist study

A single pathologist scored 20 of the 120 tissue samples for both for p53 and Ki67 (five samples randomly chosen from each scoring category) two additional times using the same PATHIAM system. Between reads, the pathologist was given a wash-out period of at least 3 days, and the samples were randomly presented each time (to further support wash-out and blinding) to the pathologist in all scoring sessions.

Test Samples for p53 Study:

Samples were sourced from a single research center, Ohio State University Medical Center (OSU), under IRB oversight and approval. 188 unique archived de-identified invasive breast carcinoma tissue specimens were used to create five TMAs. Sections of the TMAs were stained for p53 by OSU. Individual TMA cores measured 2 mm in diameter, with the total area of tissue for evaluation in each core equivalent to 16 high-power fields of view (400X magnification). The TMA slide set was scored by a qualified pathologist from BioImagene, at which time TMA cores with insufficient tissue/tumor for evaluation and/or artifacts that obscured tissue assessment were excluded. The remainder of the cores (160) were placed into one of four positivity categories: 0-1% (57), >1-5% (24), >5-10% (26) and >10% (53).

The study sample set utilized 120 total TMA cores spanning a range of positivity from 0 to 100%. The sample set of 120 consisted of all of the cores from the two middle scoring categories (>1-5%, 24 cores, and >5-10%, 26 cores). 35 cores from both the lowest and highest scoring categories (0-1% and >10%) were randomly selected from the cores in these categories, for a total of 120 cores.

TMAs were stained with the following reagents according to the procedure outlined in the table below: Antigen retrieval buffer (citrate, pH 6.0): Dako cat. No. S1699; p53: Clone DO7 (Dako cat. No. M7001); Antibody diluent, Dako cat. No. S0809; LSAB+ detection kit (Dako cat. No. K0690); DAB, (Dako cat. No. K3468).

Table: p53 Staining Procedure

Antigen retrieval with S1699 citrate buffer, pH 6.0	20-25 min
Antibody dilution using S0809	1:50
Antibody incubation	30 min at RT
Link incubation with biotinylated anti-rabbit, anti-mouse and anti-goat immunoglobulins	15 min incubation at RT
Streptavidin-Peroxidase incubation	15 min incubation at RT
DAB	5 min
Hematoxylin	15 sec

Test Samples for Ki-67 Study:
Samples were sourced from a single research center, Ohio State University Medical Center (OSU), under IRB oversight and approval. 188 unique archived de-identified invasive breast carcinoma tissue specimens were used to create five TMAs. Sections of the TMAs were stained for Ki67 by OSU. Individual TMA cores measured 2 mm in diameter, with the total area of tissue for evaluation in each core equivalent to 16 high-power fields of view (400X magnification). The TMA slide set was scored by a qualified pathologist from BioImagene, at which time TMA cores with insufficient tissue/tumor for evaluation and/or artifacts that obscured tissue assessment were excluded. The remainder of the cores (168) were placed into one of four positivity categories: 0-1% (15), >1-5% (29), >5-10% (35) and >10% (89).

The study sample set utilized 120 total TMA cores spanning a range of positivity from 0 to 100%. The sample set of 120 consisted of all of the cores from the three lower scoring categories (0-1%, >1-5%, and >5-10%) and 41 cores from the highest scoring category (>10%), which were randomly selected from the 89 cores in this category. At least 29 samples were included from all of the positivity categories with the exception of the 0-1% category. Because Ki67 is a proliferation marker and proliferation rates in breast cancer specimens are typically greater than zero, a smaller number of samples (15) from the 0-1% category were present in the TMA slide set, all of which were included in the study.

TMAs were stained with the following reagents according to the procedure outlined in the table below: Antigen retrieval buffer (citrate, pH 6.0): Dako cat. No. S1699; Ki-67: Clone

MIB1 (Dako cat. No. M7240); LSAB+ detection kit (Dako cat. No. K0679); DAB, (Dako cat. No. K3468).

Table: Ki67 Staining Procedure

Antigen retrieval	20-25 min
Antibody dilution	1:150
Antibody incubation	30-60 min at RT
Link incubation	15 min incubation at RT
Streptavidin-Peroxidase incubation	15 min incubation at RT
DAB	5 min
Hematoxylin	15 sec

Evaluations:

Comparative Study with Manual Microscopy:
For the comparative assessment, samples were scored by each investigator manually. After a minimum of one week had passed, the investigators scored the cases again using the PATHIAM system. Scoring was semi-quantitative using four percent positivity categories of 0-1%, >1-5%, >5-10%, and >10%. PATHIAM quantitative scores were also recorded.

Reproducibility:
The PATHIAM scores from the equivalence assessment were also used to assess Inter-Pathologist reproducibility.

One of the investigators for both p53 and Ki-67 (Dr. Goldstein) scored a subset of 20 cases on the PATHIAM system two additional times for the assessment of intra-reader reproducibility. A minimum of three days' washout occurred between PATHIAM scoring sessions. Additionally, the order the slides were reviewed was randomized between each scoring session.

Analytical Specificity:
The specificity of the test results is dependent on the analytical performance of the immunohistochemical staining of the tissue.

Assay Cut Off:
Clinical cut-offs used for the assessment of p53 & Ki67 varies between laboratories. The performance of the PATHIAM system was evaluated at three commonly used clinical cut-offs: >1%, >5% and >10%.

Data Collection, Data Entry, Data Verification, and Query Resolution:

Data Collection

Participating pathologists captured the scoring data on Scoring Case Report Forms. They either entered the data directly onto the forms or dictated the results to a recorder. In all cases, the investigators reviewed the data on the form and signed each page.

Data Entry Verification

Once all data entry was completed, data QC was performed. A single individual or a two person team performed data QC. When a Two Person team was available, Person 1 read the data from the original CRF while person 2 verified the data on the spreadsheet. Corrections to the data were made as needed. Comments were entered describing any changes made. The spreadsheet was designated QC in the title, and the date of the QC noted in the header of each page.

Query Resolution

Query resolution can arise from the data analysis process. Queries were sent to the investigative center in written fax or e-mail form. Corrections were made on the query resolution form, which was signed by the investigator. The forms were faxed or e-mailed back to the study coordinator, with the original also forwarded to the study coordinator. A copy was retained on site. Changes to the excel spreadsheet were noted as comments on the QC version of the file. When query resolution was complete, the final excel spreadsheet was saved as "Final". The final spreadsheets were used for the data analyses used to generate reports for regulatory submissions.

PATHIAM System Traceability

One PATHIAM System was used for this comparison study (to accommodate different study sites, two monitors were used). The details of the system are as follows:

Computer	Lenovo ThinkPad T-series 6460EGU	S/N: L3-R5511 08/09
BROWSER	Internet Exp 7 Version 7.0.5730.11	n/a
Monitor 1	Dell 2407WFPB	S/N: MX-OGM5O4-T4262-7BL-2765
Monitor 2	Dell 2407WFPB	SN: MX-0G283H-74262-92C-35LS
PATHIAM Software	PS-000617 Rev. A (V 3.1)	n/a
iScan – Scanner Software	PS-000322 Rev. Q (V 2.1.0.2)	S/N: BIO8N0059

Data Analysis

Data was analyzed for the comparative performance of manual versus PATHIAM-assisted scoring of Ki67 & p53, and for inter-reader and intra-reader reproducibility of the PATHIAM-assisted method of scoring.

Concordance ranges are presented in tables along with upper and lower 95% confidence interval ranges. Concordance calculations were determined by dividing the total number of cases with matching scores ("true" positives and negatives) by the total number of cases scored.

p53 Data Analysis and Tables

Table 18: Concordance Results for p53 Scoring

p53 Cut-Off Threshold	Manual vs PATHIAM-assisted Substantial Equivalence Concordance Range for 3 Pathologists	PATHIAM-assisted vs PATHIAM-assisted Reproducibility Concordance Range for 3 Pathologists	Manual vs Manual Reproducibility Concordance Range for 3 Pathologists
>1%	82% - 90%	88% - 93%	78% - 95%
>5%	77% - 85%	90% - 93%	78% - 88%
>10%	83% - 89%	93% - 97%	86% - 90%

Table 19: Concordance Results for p53 Scoring - Exact 95% Upper Confidence Limits

p53 Cut-Off Threshold	Manual vs PATHIAM-assisted Substantial Equivalence Concordance Range for 3 Pathologists	PATHIAM-assisted vs PATHIAM-assisted Reproducibility Concordance Range for 3 Pathologists	Manual vs Manual Reproducibility Concordance Range for 3 Pathologists
>1%	88% - 95%	93% - 97%	85% - 98%
>5%	84% - 91%	95% - 97%	85% - 93%
>10%	89% - 94%	97% - 99%	92% - 95%

Table 20: Concordance Results for p53 Scoring - Exact 95% Lower Confidence Limits

p53 Cut-Off Threshold	Manual vs PATHIAM-assisted Substantial Equivalence Concordance Range for 3 Pathologists	PATHIAM-assisted vs PATHIAM-assisted Reproducibility Concordance Range for 3 Pathologists	Manual vs Manual Reproducibility Concordance Range for 3 Pathologists
>1%	73% - 83%	81% - 87%	69% - 89%
>5%	69% - 77%	83% - 87%	70% - 80%
>10%	75% - 82%	87% - 92%	78% - 83%

Table 21: Reproducibility Concordance for Intra-Pathologist Scoring of p53

Cut-Off Threshold	PATHIAM-assisted vs. PATHIAM-assisted Reproducibility Concordance for 3 Scoring Events
>1%	85%
>5%	80%
>10%	80%

Ki-67 Data Analysis & Tables:

Table 22: Concordance Results for Ki-67 Scoring

Ki-67 Cut-Off Threshold	Manual vs. PATHIAM-assisted Substantial Equivalence Concordance Range for 3 Pathologists	PATHIAM-assisted vs. PATHIAM-assisted Reproducibility Concordance Range for 3 Pathologists	Manual vs. Manual Reproducibility Concordance Range for 3 Pathologists
>1%	88%-93%	92%-94%	86%-91%
>5%	87%-93%	90%-93%	85%-89%
>10%	81%-89%	88-95%	80%-91%

Table 23: Concordance Results for Ki-67 Scoring - Exact 95% Upper Confidence Limits

Ki-67 Cut-Off Threshold	Manual vs. PATHIAM-assisted Substantial Equivalence Concordance Range for 3 Pathologists	PATHIAM-assisted vs. PATHIAM-assisted Reproducibility Concordance Range for 3 Pathologists	Manual vs. Manual Reproducibility Concordance Range for 3 Pathologists
>1%	93%-97%	96%-98%	92%-95%
>5%	92%-97%	95%-97%	91%-94%
>10%	87%-94%	93%-98%	87%-95%

Table 24: Concordance Results for Ki-67 Scoring - Exact 95% Lower Confidence Limits

Ki-67 Cut-Off Threshold	Manual vs PATHIAM-assisted Substantial Equivalence Concordance Range for 3 Pathologists	PATHIAM-assisted vs PATHIAM-assisted Reproducibility Concordance Range for 3 Pathologists	Manual vs Manual Reproducibility Concordance Range for 3 Pathologists
>1%	81%-87%	85%-88%	78%-84%
>5%	79%-86%	83%-87%	77%-82%
>10%	73%-82%	80%-89%	72%-84%

Table 25: Reproducibility Concordance for Intra-Pathologist Scoring of Ki-67

Cut Off Threshold	PATHIAM-assisted vs. PATHIAM-assisted Reproducibility Concordance for 3 Scoring Events
>1%	80%
>5%	85%
>10%	85%

Treatment of Samples Rejected for Quality by the PATHIAM system: No cases were rejected.

End Points:
Concordance was examined at three clinically accepted standards/cut-offs for positivity, >1%, >5%, and >10%, similar to the predicates.

Conclusion:
The criterion of ≥75% concordance between manual microscopy and PATHIAM assisted scoring for the evaluation of p53 & Ki67 was met by all three pathologists for all three clinical cut-offs evaluated.

Inter-pathologist reproducibility for three pathologists using the PATHIAM system also exceeded 75% concordance at all three clinical cut-offs. Inter-Pathologist reproducibility using the PATHIAM system was higher than Inter-Pathologist reproducibility using manual microscopy at all three clinical cut-offs, indicating that PATHIAM assisted scoring is more consistent than manual microscopy.

Intra-pathologist reproducibility for 3 scoring sessions using the PATHIAM system exceeded 75% concordance at all three clinical cut-offs.

Step by Step Scoring Procedure

Manual Scoring

Overview: the pathologist manually scores test samples in tissue microarrays on glass slides under the microscope.

Materials required:

- Sample slides
- TMA maps
- Color pictures of samples taken at low magnification to help pathologist locate specific core on glass slide
- Scoring Case Report Forms

Materials required but not provided:

- Microscope

Step-by-Step Protocol:

1. Have pathologist review the training tissue samples for each scoring group (0-1%, >1-5%, >5-10%, >10%).
2. Give pathologist the randomly ordered list of test samples to be scored along with the TMA map, the corresponding TMA slide, and the low power print out corresponding to the first test sample.
3. Pathologist will locate the correct test sample under the microscope using the TMA map and the low power image of the test sample.
4. Pathologist will then review the entire test sample under the microscope as he/she would review other histopathology specimens using various objectives and freely moving the slide to evaluate multiple fields of view to arrive at a score.
5. Pathologist will record score on the provided scoring case report form for each test sample following the review of that test sample.
6. Pathologist will repeat the process for all 120 test samples.
7. Pathologist will review and sign the scoring case report form at the end of the scoring session.

PATHIAM Assisted Scoring

Overview: the pathologist will navigate the test sample images and select the field of view (at least two per test sample) on the monitor. After selecting each FOV, the pathologist will click on the "analyze" button. The pathologist will then be presented with the score for that FOV, as well as the aggregate score for all analyzed FOVs. The pathologist will have the ability to accept or reject scores for individual FOVs as well as the aggregate score. After selecting and analyzing at least two FOVs, the pathologist will select an appropriate scoring category for the sample on the case report form (which may or may not correspond to the PATHIAM aggregated score). The pathologist will also record the PATHIAM aggregated raw score for each sample.

Materials required:

- PATHIAM installed on computer
- Monitor
- Mouse, keyboard
- TMA maps
- Color pictures of samples taken at low magnification to help pathologist locate specific core on digitized slides
- Scoring Case Report Forms

Materials required but not provided: none

Step-by-Step Protocol:

1. Pathologist will open PATHIAM and login using the provided username and password.
2. Pathologist will open the case list and select case number corresponding to the first test sample on the case report form.
3. Pathologist will open the digital image and navigate to the first test sample using the TMA map. The test sample to be scored in each image will be outlined by a red box. Pathologist will select at least two fields of view (FOV) containing representative areas of the tumor for scoring. After selecting each FOV, the pathologist will click on the "analyze" button. The pathologist will then be presented with the score for that FOV, as well as the aggregate score for all analyzed FOVs. The pathologist will have the ability to accept or reject scores for individual FOVs as well as the aggregate score. After selecting and analyzing at least two FOVs, the pathologist will select an appropriate scoring category for the sample on the case report form (which may or may not correspond to the PATHIAM aggregated score). The pathologist will also record the PATHIAM aggregated raw score for each sample.
4. Pathologist will repeat the process for all 120 test samples.
5. Pathologist will review and sign the scoring case report form at the end of the scoring session.

PATHIAM System Precision/Reproducibility Studies (intra and inter system)

Title: Intersystem and intra-system performance of the PATHIAM System for analysis of Ki-67 & p53 nuclear protein immunohistochemistry in breast carcinoma tissue.

Objective:
The objectives for this study are to understand the intra system and inter system performance characteristics of the PATHIAM System for p53 and Ki-67 stained breast carcinoma tissue slides.

Sample Procurement Center:
Ohio State University Medical Center
310 Doan Hall, 410 West 10th Av, Columbus, OH
CLIA # 36D1046162

Investigators and Study Sites: The PATHIAM system study was performed at BioImagene under the supervision of Dr. Robert Monroe, Chief Medical Officer of BioImagene.

References: TP-000048 & TP-000049 Pathiam System Study Protocols for p53 & Ki-67 (breast), inter system and intra system studies.

Device Description:
The PATHIAM™ System is an instrument and software system designed to assist the qualified pathologist in the consistent quantitative assessment of protein expression in immunohistochemically stained histologic sections from formalin-fixed, paraffin-embedded normal and neoplastic tissues. The system consists of a slide scanner (iScan), computer, monitor, keyboard, mouse, image analysis algorithms for specific immunohistochemical markers, and software with a Windows web browser-based user interface. PATHIAM is a web-based, end-to-end digital pathology software solution that allows pathology labs to acquire, manage, view, analyze, share, and report on digital images of pathology specimens. Using the PATHIAM software, the pathologist can view digital images, add annotations, make measurements, perform image analysis, and generate reports.

Study Design

The intra system (PATHIAM system with iScan) study was performed on five sets of images (one set = eight test samples) produced by one scanner and scored on one computer system (consisting of a computer, monitor, keyboard, p53 & Ki-67 image analysis algorithms, MS Windows web browser and a mouse). Pre-selected field of views (8) from TMA cores randomly selected by a qualified pathologist from the four scoring categories were used for this study. Pre-selection of FOVs was necessary to allow the study scientist to locate corresponding FOVs on the multiple digital images generated by the same scanner for scoring. The goal of the inter system study was to assess the consistency and reproducibility of the PATHIAM system (no pathologist) for p53 & Ki-67 scoring on different systems.

This study was repeated on a total of three different scanners and computer systems. The same pre-selected field of views used for the inter system studies were also used for intra system study.

The test sample selection process for the system studies was as follows:
- 120 de-identified test samples were selected for the clinical studies (from the comparison studies above for p53 and Ki-67)
- These 120 test samples were designated by the TMA number and the core position.
- The core position was assigned on the basis of the row (labeled as A-E) and the column (numbered from 1-9)
- Semi-quantitative scores (0-1%, >1-5%, >5-10%, and >10%) based on prior manual microscopic review by a qualified pathologist were available for the 120 test samples
- The TMA slides containing the 120 test samples were digitized by the iScan/PATHIAM system described in detail in the report
- The digitized TMA slides/images were uploaded into the PATHIAM software
- Five test samples from each scoring category (0-1%, >1-5%, >5-10%, and >10%) were randomly selected for the intra pathologist reproducibility study being conducted in parallel
- Two of five test samples from each scoring category (total of 8) from the intra pathologist reproducibility studies were then randomly selected for the intra and inter system studies
- The digital images containing these 8 test samples were reviewed in the PATHIAM system by a qualified pathologist, with one Field of View (FOV) selected for each test sample
- The selected FOVs from the eight cores represented the area used for image analysis for both intra- and inter-system studies

Precision/Reproducibility Study results (intra and inter system)

Each test sample (8 of them) was scanned 5 times on one scanner. Each test sample (FOV) on each scan was scored by PATHIAM (raw score). The PATHIAM raw scores were used for data comparison analysis.

This was repeated on three different PATHIAM Systems (including one study from above) and scores are compared and presented in the tables below along with the PATHIAM System identification traceability information.

P53 Data Analysis

Table 26: System Identification Traceability

	SYSTEM I	SYSTEM II	SYSTEM III
Computer # Details	DELL PRECISION 5400 INTEL XEON CPU e5410 S/N 6XBQBTH1	DELL PRECISION 5400 INTEL XEON cpu e5410 S/N 9G90PJ	DELL PRECISION 5400 INTEL XEON cpu e5410 S/N DMYWHHI
Monitor # Details	DELL 24" HIGH RESOLUTION LCD S/N 262 923 195S	DELL 24" HIGH RESOLUTION LCD S/N 262 930 ID35	DELL 24" HIGH RESOLUTION LCD S/N 262 84F 1FOS
Scanner # Details	BIOIMAGENE ISCAN 2.1.0.2 (PS-000322) S/N BIO8N0071	BIOIMAGENE ISCAN 2.1.0.2 (PS-000322) S/N BIO8N0089	BIOIMAGENE ISCAN 2.1.0.2 (PS-000322) S/N BIO8N0051
Mouse	DELL DPIN OXN 967 10401GUS	DELL DPIN OXN 967 10401GUS	DELL DPIN OXN 967 10401GUS
Pathiam Software	Version 3.1, MS Browser 6.0.29	Version 3.1, MS Browser 6.0.29	Version 3.1, MS Browser 6.0.29
Keyboard	DELL SK8115 E145614	DELL SK8115 E145614	DELL SK8115 E145614

Table 27:p53 System Precision Study SYSTEM I (intra system)

P53 Precision Study – System 1 with BIO8N0071 (n=5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A7	0.00	0.00	-
TMA 3 2007	E3	0.00	0.00	-
TMA 3 2007	C9	42.90	0.02	0.06
TMA 4 2007	B5	2.82	0.08	2.67
TMA 5 2007	E3	73.50	0.05	0.07
TMA 1 2007	B9	16.44	0.01	0.09
TMA 4 2007	D4	22.14	0.07	0.32
TMA 4 2007	B3	24.05	0.06	0.23

Table 28: p53 System Precision Study SYSTEM II (intra system)

P53 Precision Study – System 2
BIO8N0089 (n=5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A7	0.00	0.00	-
TMA 3 2007	E3	0.00	0.00	-
TMA 3 2007	C9	42.74	0.02	0.05
TMA 4 2007	B5	2.57	0.01	0.58
TMA 5 2007	E3	72.89	0.04	0.06
TMA 1 2007	B9	16.51	0.04	0.24
TMA 4 2007	D4	22.44	0.04	0.17
TMA 4 2007	B3	22.68	0.06	0.25

Table 29: p53 System Precision Study SYSTEM III (intra system)

Precision Study – System 3
BIO8N0051 (n=5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A7	0.00	0.00	-
TMA 3 2007	E3	0.00	0.00	-
TMA 3 2007	C9	42.60	0.05	0.11
TMA 4 2007	B5	2.71	0.02	0.78
TMA 5 2007	E3	74.07	0.13	0.18
TMA 1 2007	B9	16.49	0.03	0.18
TMA 4 2007	D4	24.42	0.01	0.05
TMA 4 2007	B3	24.90	0.10	0.40

Table 30: p53 Inter-system Reproducibility Study – Results from System I, II, III above

Inter-System Reproducibility - System 1, 2, 3 (n=3x5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A7	0.00	0.00	-
TMA 3 2007	E3	0.00	0.00	-
TMA 3 2007	C9	42.75	0.13	0.30
TMA 4 2007	B5	2.70	0.12	4.32
TMA 5 2007	E3	73.49	0.50	0.68
TMA 1 2007	B9	16.48	0.04	0.25
TMA 4 2007	D4	23.00	1.05	4.55
TMA 4 2007	B3	23.88	0.95	3.97

Ki-67 Data Analysis

	SYSTEM I	SYSTEM II	SYSTEM III
Computer # Details	DELL PRECISION 5400 INTEL XEON cpu e5410 S/N 6XBQBTH1	DELL PRECISION 5400 INTEL XEON cpu e5410 S/N 9G90PJ	DELL PRECISION 5400 INTEL XEON cpu e5410 S/N DMYWHHI
Monitor # Details	DELL 24" HIGH RESOLUTION LCD S/N 262 923 195S	DELL 24" HIGH RESOLUTION LCD S/N 262 930 ID35	DELL 24" HIGH RESOLUTION LCD S/N 262 84F 1FOS
Scanner # Details	BIOIMAGENE ISCAN 2.1.0.2 (PS-000322) S/N BIO8N0071	BIOIMAGENE ISCAN 2.1.0.2 (PS-000322) S/N BIO8N0089	BIOIMAGENE ISCAN 2.1.0.2 (PS-000322) S/N BIO8N0051
Mouse	DELL DPIN OXN 967 10401GUS	DELL DPIN OXN 967 10401GUS	DELL DPIN OXN 967 10401GUS
Pathiam Software	Version 3.1, MS Browser 6.0.29	Version 3.1, MS Browser 6.0.29	Version 3.1, MS Browser 6.0.29
Keyboard	DELL SK8115 E145614	DELL SK8115 E145614	DELL SK8115 E145614

Table 31: Ki-67 System Precision Study SYSTEM I (intra system)

Ki67 Precision Study - System 1
BIO8N0071 (n=5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A2	31.78	0.10	0.31
TMA 3 2007	E2	64.53	0.25	0.39
TMA 3 2007	A3	15.45	0.15	0.99
TMA 4 2007	D4	17.82	0.09	0.50
TMA 3 2007	E7	9.76	0.02	0.22
TMA 5 2007	D6	4.85	0.02	0.40
TMA 3 2007	E5	9.13	0.12	1.35
TMA 2 2007	A1	0.88	0.02	1.78

Table 32:Ki-67 System Precision Study SYSTEM II (intra system)

Ki67 Precision Study - System 2
BI08N0089 (n=5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A2	32.77	0.37	1.13
TMA 3 2007	E2	63.29	0.08	0.12
TMA 3 2007	A3	15.76	0.17	1.09
TMA 4 2007	D4	17.91	0.04	0.23
TMA 3 2007	E7	9.41	0.04	0.44
TMA 5 2007	D6	4.87	0.14	2.90
TMA 3 2007	E5	9.27	0.04	0.42
TMA 2 2007	A1	0.85	0.01	0.89

Table 33: Ki-67 System Precision Study SYSTEM III (intra system)

Ki67 Precision Study - System 3
BI08N0051 (n=5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A2	31.53	0.19	0.59
TMA 3 2007	E2	62.11	0.23	0.36
TMA 3 2007	A3	15.05	0.12	0.78
TMA 4 2007	D4	17.66	0.02	0.14
TMA 3 2007	E7	9.81	0.07	0.72
TMA 5 2007	D6	4.95	0.03	0.68
TMA 3 2007	E5	9.43	0.02	0.24
TMA 2 2007	A1	0.86	0.00	0.35

Table 34: Ki-67 Inter-system Reproducibility Study – Results from System I, II, III above

Ki67 Inter-System Reproducibility - System 1, 2, 3 (n=3x5)

Line Item #	Sample ID	Mean	SD	%CV
TMA 3 2007	A2	32.03	0.60	1.87
TMA 3 2007	E2	63.31	1.04	1.65
TMA 3 2007	A3	15.42	0.33	2.14
TMA 4 2007	D4	17.79	0.12	0.66
TMA 3 2007	E7	9.66	0.19	1.95
TMA 5 2007	D6	4.89	0.09	1.84
TMA 3 2007	E5	9.28	0.14	1.53
TMA 2 2007	A1	0.86	0.02	2.07

Discussion:
The field of view analyzed for each test sample was manually drawn with the drawing tool and is therefore not exactly the same for every image analyzed in the intra & inter system studies. The variability in the reproducibility results can therefore be mostly attributed to the slight variations in the composition of the fields of view in for each image analyzed.

Conclusion:
The above tables for the intra- and inter-system studies confirm the precision and reproducibility of Ki-67 and p53 scoring within the same system and between different systems. The precision and reproducibility study data (averages, standard deviation and % CV) showed that PATHIAM System precision and reproducibility is similar to that of the predicate devices, and is therefore acceptable.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
10903 New Hampshire Avenue
Document Mail Center – WO66-0609
Silver Spring, MD 20993-0002

Bioimagene, Inc.
c/o Mr. Indu P. Lakshman
Director of Quality and Regulatory Affairs
919 Hermosa Court
Sunnyvale, CA 94085

OCT 27 2010

Re: k092333
Trade/Device Name: PATHIAM™ System with iScan for p53 and Ki67
Regulation Number: 21CFR§864.1860
Regulation Name: Immunohistochemistry reagents and kits
Regulatory Class: Class II
Product Code: NQN
Dated: September 13, 2010
Received: September 15, 2010

Dear Mr. Lakshman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical

device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please go to http://www.fda.gov/AboutFDA/CentersOffices/CDRH/CDRHOffices/ucm115809.htm for the Center for Devices and Radiological Health's (CDRH's) Office of Compliance. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Maria M Chan

Maria M. Chan, Ph.D.
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number: k092333

Device Name: PATHIAM™ System with iScan for p53 and Ki67

Indications For Use:

OCT 27 2010

Device Name: iScan Slide Scanner

Intended Use
This device is intended for in vitro diagnostic (IVD) use.

The PATHIAM System is intended as an aid to the pathologist to detect, count, and classify cells of clinical interest based on recognition of cellular objects of particular color, size, and shape, using appropriate controls to assure the validity of the scores.

Indications for Use
This instrument is intended for in-vitro diagnostic use only with those assays for which it has received FDA clearance.

The iScan Slide Scanner System is designed to be used to scan and digitize microscope slides, and compress and view digitized images of microscope slides.

If the Scanner is used in any way not specified in this manual, the protection provided by the equipment may be compromised.

Prescription Use ___X___ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) K092333

Indications for Use

OCT 27 2010

510(k) Number: k092333

Device Name: PATHIAM™ System with iScan for p53 and Ki67

Indications For Use:

Device Name: PATHIAM System with iScan for Ki-67

Intended Use
This device is intended for in vitro diagnostic (IVD) use.

The PATHIAM System is intended as an aid to the pathologist to detect, count, and classify cells of clinical interest based on recognition of cellular objects of particular color, size, and shape, using appropriate controls to assure the validity of the scores.

The Ki-67 application is intended as an aid to the pathologist to quantify the percentage of positively stained nuclei in formalin-fixed paraffin embedded normal and neoplastic breast tissue specimens immunohistochemically stained with Dako mouse monoclonal anti-human Ki-67 antigen, clone MIB1 visualized with DAB chromogen as specified in the instructions for these reagents. It is the responsibility of a qualified pathologist to employ appropriate morphological studies and controls as specified in the instructions for Dako Ki-67 to assure the validity of the PATHIAM-assisted Ki-67 assessment.

Indication For Use
Ki-67 results provided by the PATHIAM System are indicated for use to assess proliferative activity when used with in vitro diagnostic reagents marketed for this indication. Interpretation should be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. The pathologist must verify agreement with the PATHIAM score.

Prescription Use ___X___ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 807 Subpart C)

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Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) K092333

Indications for Use

510(k) Number: k092333

OCT 27 2010

Device Name: PATHIAM™ System with iScan for p53 and Ki67

Indications For Use:

Device Name: PATHIAM System with iScan for p53

Intended Use
This device is intended for in vitro diagnostic (IVD) use.

The PATHIAM System is intended as an aid to the pathologist to detect, count, and classify cells of clinical interest based on recognition of cellular objects of particular color, size, and shape, using appropriate controls to assure the validity of the scores.

The p53 application is intended for use as an aid to the pathologist to quantify the percentage of positively stained nuclei in formalin fixed paraffin embedded breast tissue specimens stained with Dako mouse monoclonal anti-human p53 antibody, clone DO7and visualized with DAB chromogen, to detect both wild-type and mutant p53, a nuclear protein, as specified in the instructions for these reagents. It is the responsibility of a qualified pathologist to employ appropriate morphological studies and controls as specified in the instructions for Dako p53 to assure the validity of the PATHIAM-assisted p53 assessment.

Indication For Use
The p53 results provided by the PATHIAM System are indicated for use for the identification of p53 accumulation in human neoplasias when used with IVD reagents marketed for this indication. Interpretation should be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. The pathologist must verify agreement with the PATHIAM score.

Prescription Use ___X___ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 807 Subpart C)

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